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Distribution of Intrinsic Plasmid Replicase Genes and Their Association with Carbapenem-Hydrolyzing Class D β-Lactamase Genes in European Clinical Isolates of *Acinetobacter baumannii*▽

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Ninety-six genetically diverse multidrug-resistant clinical isolates of *Acinetobacter baumannii* from 25 hospitals in 17 European countries were screened by PCR for specific carbapenemase-hydrolyzing class D β-lactamase (CHDL) genes and by PCR-based replicon typing for the presence of 19 different plasmid replicase (*rep*) gene homology groups (GRs). Results were confirmed by DNA sequencing where necessary. All 96 isolates contained at least 1 (with a maximum of 4) of the 19 groups of *rep* genes. Groups detected were GR6 (*rep*Aci6; 93 isolates), GR2 (including *rep*Aci1 [67 isolates] and *rep*Aci2 [3 isolates]), GR16 (*rep*ApAB49; 12 isolates), GR12 (p2ABSDF0001; 10 isolates), GR3 (*rep*Aci3; 4 isolates), GR4 (*rep*Aci4; 3 isolates), GR10 (*rep*AciX; 1 isolate), and GR14 (*rep*p4AYE; 1 isolate). Variations in *rep* gene content were observed even among epidemiologically related isolates. Genes encoding OXA-58-like CHDLs (22 isolates) were associated with carriage of the *rep*Aci1, *rep*Aci3, *rep*Aci4, and *rep*AciX genes, genes encoding OXA-40-like CHDLs (6 isolates) were associated with *rep*Aci2 and p2ABSDF0001, and genes encoding OXA-23-like CHDLs (8 isolates) were associated with *rep*Aci1. Most intrinsic *Acinetobacter* plasmids are non-self-transferable, but the almost ubiquitous *rep*Aci6 gene was strongly associated with a potential *tra* locus that could serve as a general system for plasmid mobilization and consequent horizontal transmission of plasmids and their associated antibiotic resistance genes among strains of *A. baumannii*.

Health care-associated infection with *Acinetobacter baumannii* is a rapidly increasing problem worldwide (9, 22, 23, 29). For several decades, large proportions of *A. baumannii* isolates from health care facilities have exhibited resistance to most commonly used antibiotics, including aminopenicillins, ureidopenicillins, broad-spectrum cephalosporins, most aminoglycosides, quinolones, tetracyclines, and chloramphenicol (3, 20, 22, 33). As a consequence, carbapenems (especially imipenem and meropenem) have been the mainstay of treatment for *Acinetobacter* infections. However, reports of resistance to carbapenems have accumulated worldwide, with some isolates now being resistant to all conventional antibiotics (8, 10, 19, 25, 30). *A. baumannii* seems to be particularly adept at acquiring and expressing new mechanisms of resistance in response to challenge with novel antibiotics. It is therefore important to understand the mechanisms of acquisition of resistance genes by *A. baumannii* and to elucidate the rate and nature of genetic exchanges.

Comparative genomic analysis of a small number of clinical *A. baumannii* isolates previously revealed a poor correlation between genetic relatedness and patterns of antimicrobial susceptibility (1, 17, 35). Furthermore, a study involving whole-genome sequence analysis of six closely related clinical isolates revealed extensive divergence of the resistance genotype, with resistance genes associated with insertion sequences, plasmids, and a chromosomal resistance gene island all showing extensive variability, suggesting rapid evolution of drug resistance (2). Although a number of different mechanisms of resistance to carbapenems have been reported for *Acinetobacter* spp. (25), most clinically significant carbapenem resistance in this species has been associated with plasmid-mediated acquisition of genes encoding either class B metallo-β-lactamases or carbapenem-hydrolyzing class D OXA-type β-lactamases (CHDLs), with CHDLs representing the most important and widespread mechanism of carbapenem resistance (25). Four major groups of acquired CHDLs have been identified in *A. baumannii*, represented by OXA-23, OXA-40, OXA-58, and OXA-143 (16, 24). These acquired CHDL genes often remain plasmid carried but can also become integrated into the bacterial chromosome (24), perhaps under antibiotic selection pressure following plasmid vector instability. In addition, all isolates of *A. baumannii* possess an intrinsic chromosomally located $bla_{\text{OXA-51-like}}$ CHDL-encoding gene that is capable of reducing susceptibility to carbapenems when it is overexpressed in conjunction with a promoter supplied by an upstream insertion sequence, IS*Aba*1 (11, 12, 32). Although originally chromosomally located and found solely in *A.*

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baumannii, $bla_{OXA\text{-}51\text{-}like}$ CHDL-encoding genes linked to IS*Aba*1 have now also been reported to be carried on plasmids isolated from several different *Acinetobacter* spp. in Taiwan, including *A. baumannii*, and have been associated directly with high levels of carbapenem resistance (6).

Plasmids are notoriously difficult to isolate and study in *Acinetobacter* spp. However, an early study in 1985 with the genus *Acinetobacter* (predating the delineation of *A. baumannii* as a separate species) revealed that many different plasmid incompatibility groups found in the *Enterobacteriaceae* are capable of transfer from *Escherichia coli* to the *Acinetobacter* genus (7). However, the subsequent behavior of enterobacterial plasmids in *Acinetobacter* (stability, retransfer, ability to be mobilized, etc.) varied greatly and was influenced by the presence/absence of intrinsic plasmids in the strain of *Acinetobacter* investigated (7). In the subsequent 25 years, no extensive surveys have been published concerning the distribution and epidemiology of particular intrinsic plasmid types in *Acinetobacter* spp., although analysis of the fingerprint size profiles of intrinsic plasmids has been proposed as a typing method for clinical isolates of *Acinetobacter* spp. (27, 28). However, sequence analysis has indicated that *A. baumannii* plasmid replicons differ from all those described previously for other prokaryotic species, indicating that *A. baumannii* possesses its own complement of distinct plasmid types (5). The development of a comprehensive PCR-based replicon typing method has now provided a new tool for large-scale investigations of the epidemiology of intrinsic plasmids in *A. baumannii*. The aim of the present study was to exploit the new multiplex replicon typing system (5) to examine the distribution and epidemiology of intrinsic plasmid replicase (*rep*) genes in 96 genotypically diverse clinical isolates of *A. baumannii* from 25 hospitals in 17 European countries. In addition, the potential association of individual plasmid *rep* gene groups with the horizontal dissemination of genes encoding CHDLs among *A. baumannii* strains was investigated.

MATERIALS AND METHODS

Bacterial isolates. The panel of *A. baumannii* isolates used in this study comprised 96 well-characterized multidrug-resistant clinical isolates obtained from patients with a range of invasive infections who were hospitalized in 25 hospitals in 17 different European countries (Table 1). The isolates were collected on the basis of their reported carbapenem resistance as part of the European Union-funded Antibiotic Resistance Prevention and Control (ARPAC) project (21, 31), but they also exhibited resistance to aminopenicillins, ureidopenicillins, broad-spectrum cephalosporins, aminoglycosides, quinolones, tetracyclines, and chloramphenicol. The isolates were genotypically diverse, belonging to 17 different pulsed-field gel electrophoresis (PFGE) groups, including PFGE groups forming part of the epidemic European clonal (EC) or worldwide (WW) lineages 1, 2, and 3 (31). Multilocus sequence typing (MLST) sequence types were assigned previously to 45 of the isolates included in the panel (14), with at least 13 different MLST types being represented (Table 1). Epidemiologically related isolates (belonging to the same PFGE group) from different patients in the same hospital were included in the panel of isolates in order to examine short-term variation in plasmid *rep* gene content (Table 1).

AB-PBRT. In total, 19 PCR amplifications were used as described previously (5) to detect 27 different plasmid *rep* genes, originally identified from the published sequences of partially or fully sequenced plasmids isolated from *Acinetobacter* spp. The *A. baumannii* PCR-based replicon typing (AB-PBRT) scheme groups the 27 *rep* genes into 19 homology groups (GRs) on the basis of their nucleotide sequence similarities. These groups are then detected using six multiplex PCRs, each recognizing three or four different homology groups (Table 2).

DNA extracts were prepared from overnight nutrient broth cultures by use of a Wizard DNA extraction kit (Promega, Southampton, United Kingdom). PCRs were performed using Ready-To-Go PCR beads (GE Healthcare Life Sciences, Little Chalfont, United Kingdom), together with previously described primers (5), in a final volume of 25 μl on a Progene thermal cycler (Techne, Cambridge, United Kingdom). PCR conditions were as follows: 94°C for 5 min, 30 cycles of 95°C for 30 s, 54°C for 20 s, and 72°C for 45 s, and a final extension at 72°C for 5 min. PCR products were visualized by ethidium bromide staining following electrophoresis on 1.5% (wt/vol) agarose gels in Tris-borate electrophoresis buffer. Plasmid *rep* gene controls are listed in Table 2.

Some GRs contained more than one *rep* gene variant (Table 2). These were distinguished by purifying the amplicons with a QIAquick PCR purification kit (Qiagen, Crawley, United Kingdom), followed by sequencing in both directions on a model 3730 DNA analyzer (Applied Biosystems, Warrington, United Kingdom).

Detection of plasmid-mediated *tra* locus. An additional PCR was designed to identify the gene encoding the type IV secretory pathway VirB4 component, also called type IV secretion system protein TraC, found on plasmid pACICU2 (which also carries the *rep*Aci6 gene). Primers were designed from the published sequence (GenBank accession number CP000865.1) to amplify a 639-bp product and were as follows: forward, 5′-AACAAAGCAAGAATAAAGC-3′; and reverse, 5′-AAATCAATTGCTTGTCCTTT-3′. PCR parameters were as described above, but with an annealing temperature of 50°C.

Detection of genes encoding CHDLs. A multiplex PCR (34) was used to screen the isolates for the presence of the four main groups of CHDLs reported for *A. baumannii*, namely, the intrinsic $bla_{OXA\text{-}51\text{-}like}$ gene, until recently found only in *A. baumannii* (6, 14, 15), and the three main families of acquired CHDL genes ($bla_{OXA\text{-}23\text{-}like}$, $bla_{OXA\text{-}40\text{-}like}$, and $bla_{OXA\text{-}58\text{-}like}$). A separate PCR (16) was used to screen the isolates for the presence of the gene encoding OXA-143, which to date is the sole representative of a new subgroup of CHDLs found in *A. baumannii* isolates from Brazil (16).

RESULTS AND DISCUSSION

Distribution of intrinsic plasmid *rep* genes. All 96 isolates of *A. baumannii* investigated contained at least 1 (with a maximum of 4) of the 19 groups of plasmid *rep* genes. Table 3 summarizes the plasmid *rep* gene profiles detected, grouping the geographically related isolates that exhibited identical *rep* gene profiles. In some cases (e.g., isolates A372 and A373 and isolates A473 and A474), genotypically distinct isolates from the same hospital were found to have identical plasmid *rep* gene profiles. In contrast (e.g., the A422 group and the A425 group), isolates with the same PFGE type that were obtained from patients in the same hospital were found to have different plasmid *rep* gene profiles (Table 3), thereby indicating that these intrinsic plasmid *rep* gene profiles could be unstable in even a relatively short-term epidemiological setting. Although it is not known whether these *rep* genes always exist on autonomous plasmid replicons or whether they sometimes become integrated into the chromosome as a result of plasmid replicon instability, the apparent potential short-term instability of the intrinsic plasmid complement is in agreement with the extensive divergence of resistance genotypes revealed by whole-genome sequence analysis of six closely related clinical isolates (2).

Table 4 summarizes the relative distribution of the plasmid *rep* genes detected in the overall collection of *A. baumannii* isolates. GR6 (*rep*Aci6) was almost ubiquitous (93 of 96 isolates), followed by GR2 (*rep*Aci1 and *rep*Aci2, together found in 70 isolates). Six other *rep* gene groups were also detected, but none was found in more than 12 isolates (Table 4). With few exceptions, most intrinsic *Acinetobacter* plasmids seem to be non-self-transferable in the laboratory, a finding which has been supported by the failure of *in silico* analysis to detect putative conjugation systems in most instances (5). However, comparative analysis of plasmid and chromosome sequence

TABLE 1. Clinical isolates of *A. baumannii* included in this study ($n = 96$)[b]

Isolate(s)	Source[a]	Imipenem MIC (μg/ml)	PFGE type	EC or WW lineage	MLST type
A369, A370	Spain	128	I	2	NA
A371	Czech Republic	4	III	2	92
A372	Greece (hospital 1)	32	IV	1	NA
A373	Greece (hospital 1)	32	XVI	2	NA
A374	Netherlands	32	X	New	249
A376	Austria	32	XIII	New	NA
A377	Germany (hospital 1)	32	XI	3	187
A380, A381, A382, A383	United Kingdom	16	XVI	2	NA
A384	Norway	16	VI	1	95
A385, A387	Greece (hospital 2)	16	XVI	2	189
A386	Greece (hospital 2)	16	IV	1	NA
A388	Greece (hospital 2)	16	IV	New	248
A389	Denmark	8	XV	New	NA
A390, A391	Bulgaria (hospital 1)	16	VII	1	NA
A392	Germany (hospital 2)	64	IV	2	98
A393	Germany (hospital 2)	32	XV	2	NA
A394, A395, A396, A397, A398	Greece (hospital 3)	16	IV	2	4
A399, A400	Turkey	16	X	New	NA
A404, A405, A406, A407, A408, A409	Poland (hospital 1)	<4	VIII	1	245
A410	Poland (hospital 1)	4	XV	2	NA
A411, A412, A413	Poland (hospital 1)	<4	VII	1	NA
A414, A415	Poland (hospital 2)	4	VII	1	NA
A416, A417, A418, A419, A420, A421	Poland (hospital 2)	8	II	2	NA
A422, A423, A424, A425, A426, A427, A428, A429, A430, A431, A432, A433, A434, A435, A436, A437	Croatia	8	V	1	246
A438, A439, A440, A441, A442	Bulgaria (hospital 2)	16	IX	1	109
A443	Slovenia	8	VII	1	245
A444, A445, A446, A447, A448, A449, A450, A451, A452	Poland (hospital 3)	8	XVII	1	NA
A453, A454	Slovakia	8	III	2	NA
A457	Estonia	<4	XII	New	243
A458, A459	Estonia	8	XII	1	NA
A461, A462, A463, A464, A465, A466, A467	Portugal	8	XIV	New	NA
A468	Poland (hospital 4)	4	VIII	2	NA
A469	Poland (hospital 4)	4	XVI	1	NA
A470	Poland (hospital 4)	8	XVII	2	NA
A472	Poland (hospital 5)	8	VIII	1	245
A473	Poland (hospital 5)	4	XVI	2	252
A474	Poland (hospital 5)	4	XVII	2	NA

[a] Isolates from the same country were from a single hospital, except where indicated.
[b] MLST types shown are those now permanently allocated in the *Acinetobacter* MLST database (http://pubmlst.org/abaumannii/) and differ from those published previously (14). PFGE types and EC or WW lineages shown are as determined previously (14, 31). "New" denotes an isolate that did not belong to EC (WW) lineage 1, 2, or 3. NA, isolate has not yet been assigned to an MLST type.

data available for the genus *Acinetobacter* has revealed that the absence of mobilization and transfer functions on most acinetobacter plasmids does not seem to have posed a particular barrier to horizontal gene transfer (13).

A notable exception is the *rep*Aci6 gene. This gene was originally identified on plasmid pACICU2, which also carries a putative *tra* gene and is therefore potentially transferable (18). The *rep*Aci6 gene was also identified as the unique *rep* gene carried by four plasmids that were successfully transferred in mating experiments, thereby confirming the association of the *rep*Aci6 gene with the self-transferability property of this *A. baumannii* plasmid group (5). Further PCR analysis of the isolates in the present study that carried the *rep*Aci6 gene revealed that 88 of these 93 isolates also carried a gene encoding the type IV secretion system protein TraC. The three isolates that did not carry the *rep*Aci6 gene also lacked the gene encoding TraC. Thus, the *rep*Aci6 gene was strongly linked with the gene encoding TraC, and the almost ubiquitous occurrence of the *rep*Aci6 gene (93 of 96 clinical isolates in the present study) could therefore indicate the presence of a common plasmid with the potential to mobilize other plasmids, including plasmids carrying genes encoding a range of resistance determinants, such as CHDLs.

Distribution of genes encoding CHDLs. All 96 isolates carried a $bla_{\text{OXA-51-like}}$ gene, normally considered to be an intrinsic chromosomally located gene that is diagnostic of *A. baumannii* (6, 14, 15). The associations of plasmid *rep* genes with genes encoding acquired CHDLs are summarized in Tables 3 and 5. Genes encoding OXA-58-like CHDLs were most common (22 isolates) and were associated with carriage of *rep*Aci6, *rep*Aci1, *rep*Aci3, *rep*Aci4, and *rep*AciX. Genes encoding OXA-23-like CHDLs (8 isolates) were associated with *rep*Aci6 and *rep*Aci1. Genes encoding OXA-40-like CHDLs (6 isolates) were associated with carriage of *rep*Aci6, *rep*Aci2, and

TABLE 2. Plasmid replicon typing scheme for *A. baumannii* based on the sequences of 19 different groups of *rep* genes

Multiplex no.	Group	Amplicon size (bp)[a]	Plasmid replicase	Control strain or plasmid
1	GR1	330	p1ABSDF001	SDF
	GR3	505	Aci3 + Aci7	Ab537
	GR2	851	Aci1 + Aci2	AYE
2	GR5	220	Aci5	Ab537
	GR18	676	p2ABSDF00025	SDF
	GR7	885	p3ABSDF002	SDF
3	GR9	191	p3ABSDF0009	SDF
	GR4	508	Aci4	Ab844
	GR11	852	p1ABAYE0001	AYE
4	GR12	165	p2ABSDF0001	SDF
	GR10	371	AciX	ACICU
	GR13	780	p3ABAYE0002	AYE
5	GR8	233	Aci8 + Aci9 (RepM)	Ab11921
	GR14	622	p4ABAYE0001	AYE
	GR15	876	p3ABSDF0018	SDF
6	GR16	233	RepApAB49	pAB49
	GR17	380	A1s_3471	ATCC17978
	GR6	662	Aci6	ACICU
	GR19	815	Rep135040	Ab135040

[a] Primers used to detect individual *rep* genes were those described previously (5).

TABLE 3. Results of *rep* and CHDL gene analyses of 96 European isolates of *A. baumannii*, grouped according to *rep* gene content and geographical relationships

Isolate(s)	*rep*Aci gene content (GR no.)	CHDL content[a]
A369, A370	6, 12	OXA-40
A371	6	
A372, A373	1, 6	OXA-58
A374	1, 6	OXA-23
A376	12	
A377	2, 6	OXA-58
A380, A381, A382, A383	1, 6	OXA-23, OXA-58
A384	6, 12	OXA-58
A386	1, 10	OXA-58
A385, A387	1, 6	OXA-58
A388	1, 6	OXA-58
A389	3	OXA-58
A390, A391	1, 6	OXA-23
A392, A393	6	OXA-58
A394, A395, A396, A397, A398	1, 6	OXA-58
A399, A400	3, 6, 4, 12	OXA-58
A404, A405, A406, A407, A408, A409, A410, A411, A412, A413	1, 6	
A414, A415, A416, A417, A418, A419, A420, A421	1, 6	
A422, A423, A424, A435, A436	1, 6	
A425, A426, A427, A428, A429, A430, A431, A432, A433, A434, A437	1, 6, 16	
A438, A439, A441, A442	1, 6, 16	
A440	6	OXA-23
A443	6	
A445, A446, A447, A448, A449, A450, A451, A452	1, 6	
A453, A454	6	
A457	3, 6	
A458, A459	6	
A461	4, 6, 12, 14	OXA-58
A462, A463, A466	1, 6, 12	
A464, A465, A467	6	OXA-40
A468, A470	1, 6, 16	
A469	1, 6	
A472	1, 6	
A473, A474	6	

[a] All isolates also carried a gene encoding an intrinsic OXA-51-like CHDL.

p2ABSDF0001. None of the 96 European isolates of *A. baumannii* examined in the present study was found to carry a gene encoding OXA-143.

Carbapenem resistance in *A. baumannii* can be multifactorial, sometimes involving a combination of enzymatic and other mechanisms, such as permeability, outer membrane proteins, and penicillin-binding proteins (25). The isolates with imipenem MICs of ≥16 μg/ml, which would be regarded as resistant according to CLSI criteria, were almost exclusively (26/28 isolates [92.9%]) associated with the carriage of an acquired class D carbapenemase. However, all 96 isolates also carried an intrinsic OXA-51-type carbapenemase, whose enhanced expression has been shown to be capable of conferring a carbapenem MIC of 64 μg/ml in *A. baumannii* (6, 11, 12). It therefore seems likely that this mechanism could be responsible for resistance in the two remaining isolates with imipenem MICs of ≥16 μg/ml. Interestingly, some of the 96 isolates, which were initially selected on the basis of their resistance to carbapenems, were found to have imipenem MICs of ≤4 μg/ml (Table 1), perhaps indicating the instability of this form of resistance in the absence of continued selection pressure. Apart from carbapenem resistance, the multidrug resistance phenotype of these isolates meant that it was not possible to identify any correlation between the presence of particular intrinsic plasmid *rep* genes and the presence of other antibiotic resistance genes.

The *rep*Aci1 and *rep*Aci2 genes are found mostly on plasmids that also carry the Re27 sequence (26). Although very few *Acinetobacter* plasmids carrying genes encoding CHDLs have been sequenced fully, the 27-bp Re27 sequence seems to denote a favored insertion site for structures carrying OXA-58-like CHDL genes. For example, in strain MAD from France, the genetic structure surrounding the OXA-58 gene is bracketed by two copies of the Re27 sequence (26). Similar structures have been identified in isolates from Italy and Lebanon (4, 36), and it has been suggested that a site-specific recombi-

TABLE 4. Overview of plasmid *rep* gene distribution among 96 clinical isolates of *A. baumannii*

GR no., *rep* gene detected	No. (%) of isolates
GR6, *rep*Aci6	93 (96.8)
GR2, *rep*Aci1	67 (69.8)
GR2, *rep*Aci2	3 (3.1)
GR16, *rep*ApAB49	12 (12.5)
GR12, p2ABSDF0001	10 (10.4)
GR3, *rep*Aci3	4 (4.2)
GR4, *rep*Aci4	3 (3.1)
GR10, *rep*AciX	1 (1.1)
GR14, p4ABAYE0001	1 (1.1)

TABLE 5. Overview of association of genes encoding acquired CHDLs with plasmid *rep* gene content

Acquired CHDL gene (*n*)	Plasmid *rep* gene content (GR no.)	No. of isolates
$bla_{\text{OXA-23-like}}$ (8)	1, 6	7
	6	1
$bla_{\text{OXA-40-like}}$ (6)	2, 6, 12	2
	6	4
$bla_{\text{OXA-58-like}}$ (22)	1, 6	13
	3, 6, 4, 12	2
	6	2
	4, 6, 12, 14	1
	2, 6	1
	6, 12	1
	1, 10	1
	3	1

nation process could be involved in the acquisition of the $bla_{\text{OXA-58}}$ locus (4, 26, 36). The finding in the present study that the *rep*Aci1/*rep*Aci2 group was associated with 14 of the 22 isolates that encoded OXA-58-like CHDLs (Table 5) provides further support for this suggestion. Similar processes are probably involved in the acquisition of genes encoding OXA-23-like and OXA-40-like CHDLs. Thus, although CHDL genes were also associated occasionally with the presence of other plasmid *rep* genes, the presence of *rep*Aci1 or *rep*Aci2 not only reflects the fact that acquired CHDL genes have been reported mostly for plasmids that also carry the linked Re27 sequence but also may indicate a particular propensity for strains to acquire such genes. Furthermore, the presence of acquired CHDL genes was almost always (34 of 36 isolates) associated with *rep*Aci6, linked in turn to a potential conjugation system that could act to mobilize other plasmids present in the same cell.

Conclusions. The AB-PBRT system was found to be an easy, rapid, and reliable tool for large-scale investigations of the epidemiology of intrinsic plasmid *rep* genes in clinical isolates of *A. baumannii*. Specific *rep* genes were detected in all 96 of the genotypically diverse European clinical isolates of *A. baumannii* examined in the present study, with some isolates carrying as many as four different plasmid *rep* gene types. Genes encoding CHDLs such as OXA-58 were particularly associated with carriage of the *rep*Aci1 gene, which in turn probably reflects their linkage with the Re27 sequence, which seems to denote a favored insertion site for molecular structures carrying genes encoding CHDLs (26).

A. baumannii is well known for the speed with which it appears able to acquire and express new mechanisms of resistance in response to challenge with novel antibiotics. In this respect, the diverse nature of the intrinsic plasmid complement in this species, as indicated by the detection of different *rep* genes in the clinical isolates examined in the present study, suggests that there is an enhanced basis for genomic rearrangements and incorporation of new resistance genes, perhaps at insertion sites such as the Re27 sequence. Of particular interest was the almost ubiquitous occurrence of the *rep*Aci6 gene, which could be indicative of the presence of a general intrinsic system for plasmid mobilization and consequent horizontal transmission of foreign plasmids and their associated antibiotic resistance genes among clinical strains of *A. baumannii*. Further studies will be required to elucidate the precise genetic linkage between particular *rep* genes and individual antibiotic resistance determinants in order to elucidate the rate and nature of genetic exchanges. However, the results of the present study with clinical isolates of *A. baumannii* provide further evidence for the potential within this genus for very dynamic reorganization and flexibility of plasmid architecture under fluctuating environmental and selective conditions (13).

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